Neurofilament high molecular weight-green fluorescent protein fusion is normally expressed in neurons and transported in axons: a neuronal marker to investigate the biology of neurofilaments

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Résumé en anglais	The carboxy-terminal side arm of the neurofilament high subunit consists of a highly phosphorylated domain and a negatively charged region. Multiple evidences suggested that these domains are essential for the axonal phosphorylation and transport of neurofilaments and play a role in their abnormal accumulation following chemical intoxication or during neurodegenerative disorders such as amyotrophic lateral sclerosis. In order to investigate the consequences of altering this side arm of neurofilament high subunit we used a fusion protein (neurofilament high subunit-green fluorescent protein) between the mouse neurofilament high subunit missing a major part of the C-terminal domain and the reporter green fluorescent protein. In cell culture and in transgenic mice this fusion protein co-assembles and co-distributes with the endogenous intermediate filament network. Conditions known to disturb the cytoskeleton were also found to alter the distribution of the fusion protein in cell cultures. In transgenic mice the expression of the transgene evaluated by its fluorescent properties was found to be restricted to neurons, where the neurofilament high subunit-green fluorescent protein fusion protein is axonally transported. Biochemical approaches showed that the fusion protein is phosphorylated and co-purified with neurofilaments. Despite the presence of such an neurofilament high subunit-green fluorescent protein fusion protein, the axonal cytoskeletal density and the axonal caliber were not altered. Together these data show that removal of this portion of neurofilament high subunit does not affect the capacity of neurofilament high subunit to assemble and to be transported into axons, suggesting that this sequence is involved in another function. Moreover, the fluorescent properties of this fusion protein represent a useful marker.
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Liens

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